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14. ABSTRACT

Neural stem cells (NSC) are multipotent cells isolated from striatal tissue and the subventricular zone, which is one of a few neurogenic areas in the adult brain. Evidence suggests that NSC proliferation and differentiation occur under physiological conditions and can be enhanced in certain pathological conditions following neural damage. However, the communication between soluble factors of inflammatory T cells and NSCs that affect the proliferation and differentiation of NSCs remain unknown. The goal of this project is to develop a novel NSC culture platform that is capable of both compartmentalizing and fluidically isolating microdomains of NSCs.

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Introduction.

In certain autoimmune diseases of the central nervous system (CNS) in which regeneration does not occur, Neural Stem Cells (NSCs) fail to migrate to and proliferate at the site of damage. Migration and differentiation of NSCs have been studied *in vivo* as well as their response to factors released by activated T-cells. Recent progress in our understanding of the biology of NSCs has inspired interest in exploring the roles of neurogenesis capable NSC in the pathology and therapy of neurodegenerative disorders including multiple sclerosis (MS) [1-2]. Evidence suggests that NSC proliferation and differentiation occur under physiological conditions and can be enhanced in certain pathological conditions following neural damage [3-4]. This has largely been due to the inability to precisely control the fluidic microenvironment in standard *in vitro* preparations.

Body.

The goal of our proposal is the development of a novel microfluidic platform which can partially expose NSCs inflammatory T cell in a fluidically isolated manner. We have successfully developed a novel microfluidic compartmentalized NSC culture platform that enables culturing NSCs in multiple fluidic environments (Fig 1). NPCs migrated into and through the microchannel of the device and remained viable for 21 days, using visual inspection to determine if cells were alive. Microchannel length and well size in the lower layer were varied to determine any impact on cell survivability. Microchannels that were longer than 0.5 mm did not have high cell density due to lack of nutrients. The microchannels were limited to at least 0.5 mm due to physical difficulties fabricating smaller lengths. For well diameters less than 1.0 mm, NPCs entered the microchannel with higher probability (67%) than in the case of the 2 mm diameter (25%) by 14 days after seeding the neurosphere. Well size did not noticeably affect cell growth inside the microchannel. Fluidic isolation of fluorescent dye was achieved for 3 days using a flow of 10 microliters/hour for microchannels of cross sectional dimensions 50 microns x 50 microns. The method for examination was both visual examination by eye and examination under a fluorescence microscope.

Key research accomplishments

· **Development of microfluidic platform for NSCs culture.**
  - · Optimization of compartmentalized wells.
  - · Fluidic isolation of solution between compartmentalized wells.
  - · Successful culture of NSCs in the microfluidic device.

Reportable Outcomes

None.

Conclusion.

The device creates a system in which NPCs migrate through the microchannel within 20 days and with good survivability for multiple weeks. It also can fluidically isolate one compartment by introducing a steady but very low flow through the microchannel. The ability of the system to perform both tasks makes it suitable for a system to conduct the migration and differentiation experiments described earlier, as well as for studying cell-cell signaling in many applications.

Figure 1.

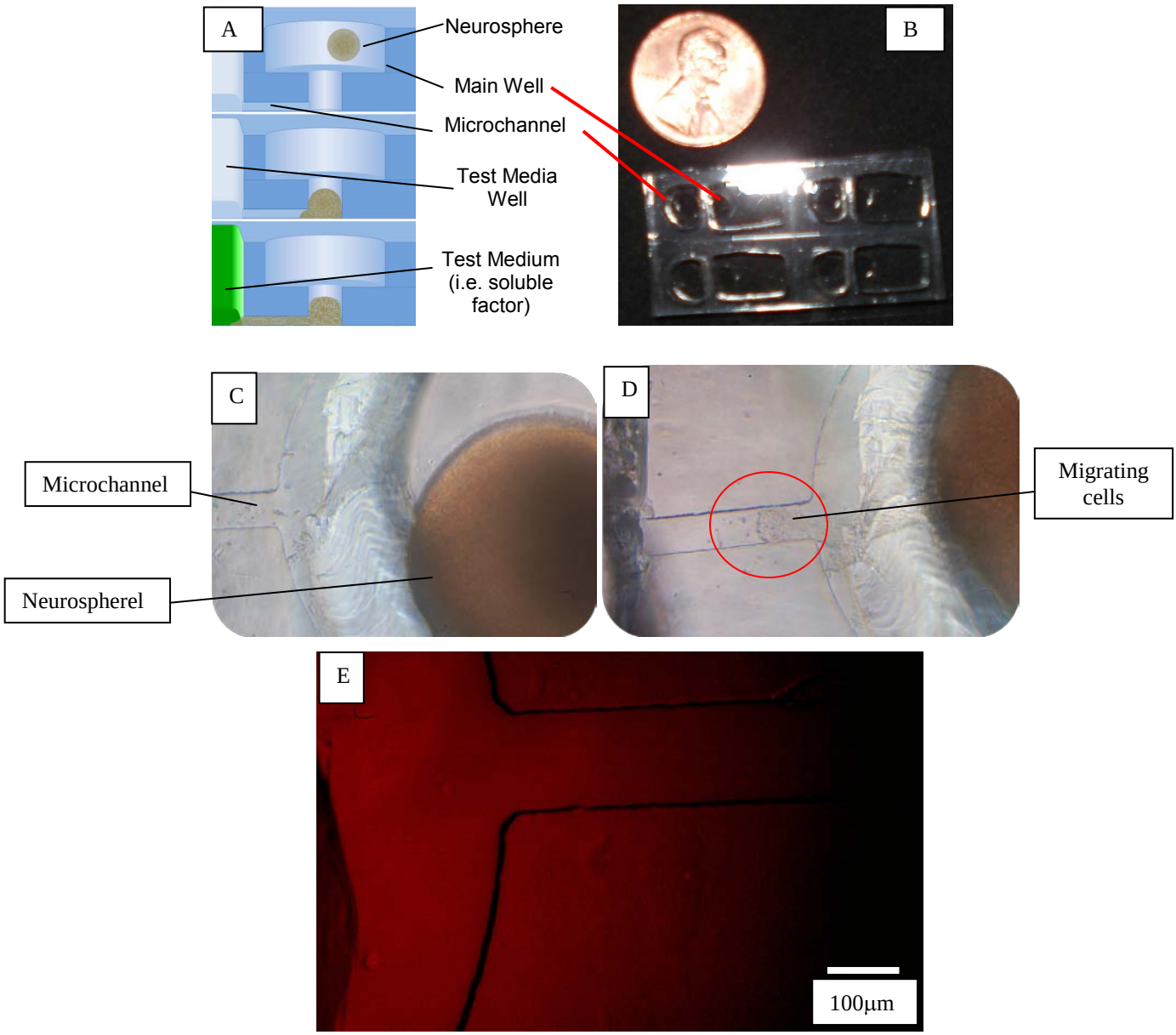

Figure 1 (A). Diagram of the device in use. A ball of NSCs is first placed in the well then allowed to settle and attach to the bottom of the well. After a period of a few days, NSCs have migrated through the microchannel to produce the desired model. (B). Picture from above the device. A main well with the NSCs has a microchannel to another well with the desired soluble factor. (C). Neurosphere (NPCs) were placed in the well in the lower PDMS layer next to the microchannel. (D). NPCs spread from neurosphere into the microchannel and continue to migrate/proliferate until NPCs reach the second well (Red Circle). (E). The fluidic isolation picture included here was not a cell culture, and a fluorescent dye was used merely to demonstrate the concept.

References.

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Appendices

None.